Peer Review
Experimental Toxicology Division
NHEERL

EMERGING ISSUES

Oxidative Mechanisms of Toxicity and Susceptibility

Gary Hatch, Ralph Slade, Joel Norwood, Kay Crissman, John McKee, Bobby Crissman

INTRODUCTION

Environmental Issue

Oxidation, similar to that encountered in the bleaching of fabric, occurs inside the body and is associated with pollutant exposure and disease:

DIRECT OXIDANT POLLUTANTS
Particulate matter (peroxides, quinones, metals)
Air pollutant gases (ozone, NO_2, Cl_2)
Bromate
Tobacco smoke

INDIRECT OXIDANTS
Metals (cadmium, vanadium, etc.)
Asbestos
Silica
Carbon tetrachloride
Dioxin
Ultraviolet light
Ionizing radiation (radon)

INTERNALLY GENERATED OXIDANTS
Injured mitochondria
Inflammation: activated phagocytes.

NORMAL AGING

DISEASES
Cancer (initiation and promotion)
Atherosclerosis
Diabetes
Asthma
Alzheimer's

Inflammatory Disease (asthma, arthritis, autoimmune)

Study Goals

1) To understand the oxidative mode of action of pollutants

2) To improve measurements of oxidative stress

Scientific Approach

"Oxidative stress" involves both electron withdrawal and oxygenation reactions.

Electron Withdrawal: RH $\xrightarrow{e^-}$ R· + H⁺

Oxygenation: "Primary" R + O· ⟶ R-O
"Secondary" R· + O_2 ⟶ R-O-O ⟶ Reaction "Cascade"

WHY IS OXYGENATION TOXIC?

It leads to FORMATION OF STRONG, IRREVERSIBLE BONDS

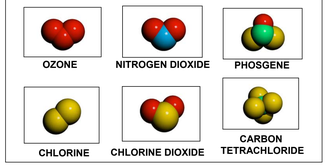
BOND ENERGY OF COMMON CHEMICAL BONDS

Bond Type	Energy, kcal/mole
C ═O	175
C — H	96
C — O	89
C — C	85
S — H	82
C — N	72

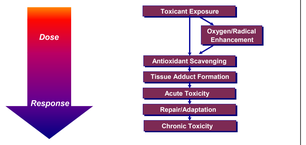
METHODS

Adduct Formation/Oxygenation

OXIDATIVE TOXICANT MOLECULES



Many toxicants exert their effects through the formation of "tissue adducts" involving oxygen or similar molecules

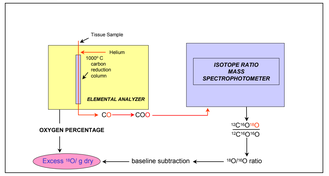


Measurement of "oxygenation" reactions

Molecules we have labeled with ^{18}O

O_2 OXYGEN	O_3 OZONE	H_2O_2 HYDROGEN PEROXIDE
$ONOO^-$ PEROXYNITRITE ANION	NO_2 NITROGEN DIOXIDE	$NO^·$ NITRIC OXIDE

MEASUREMENT OF OXYGEN-18 IN TISSUES

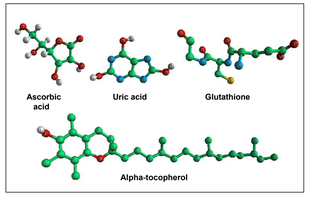


Antioxidant Measurement

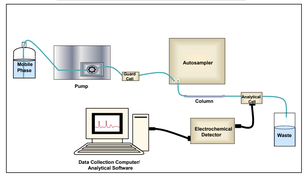
Antioxidants found in fruits and vegetables perform important protective roles in the body.

Measurement of antioxidant scavenging is done by measuring the concentration of antioxidant substances

ANTIOXIDANT SUBSTANCES ASSAYED



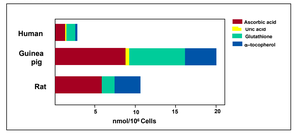
MEASUREMENT OF ANTIOXIDANTS



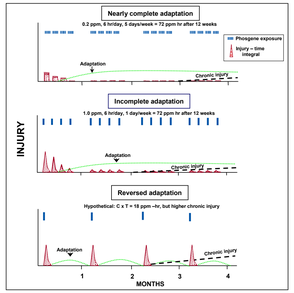
RESULTS

Recent results in Study Goal 1:
To understand the oxidative mode of action.

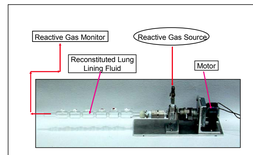
- Humans have low concentrations (compared to rodents) of two important antioxidants: glutathione and ascorbate. [1]



- Rodents depleted of antioxidants to the level of humans have increased susceptibility to both ozone and PM. [2]
- Ozone dosimetry studies suggest that 1) species with high oxygen consumption are most susceptible, and 2) these species defend against injury by lowering activity and body temperature.
- Progression of acute injury to toxic injury appears to be most severe following episodic exposures rather than continuous exposures. [3]



- A "lung uptake simulator" has been developed which allows direct modeling of oxidative interactions in airway lining fluids.



This "lung uptake simulator" is a rotating glass tube into which lung lining fluid is added. Polluted air is drawn through the tube, modeling inhalation exposures.

Reconstituted airway lining fluid is added to the lung uptake simulator to elucidate oxidant mode of action

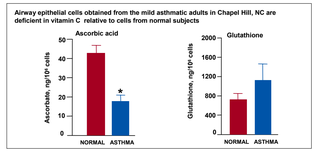
Reconstituted airway lining fluid *:

Mucin[a]	10 mg/ml
Surfactant lipid[b]	10 mg/ml
Albumin[a]	7 mg/ml
Other proteins[a]	3 mg/ml
Antioxidant substances:	
ascorbate	50 μg/ml
glutathione	50 μg/ml
urate	25 μg/ml
α-tocopherol	1 μg/ml

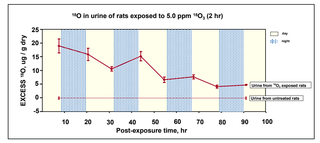
Base: Hanks balanced salt solution
* From a porcine source
[a] From a bovine source.

Recent results in Study Goal 2:
To improve measurements of oxidative stress.

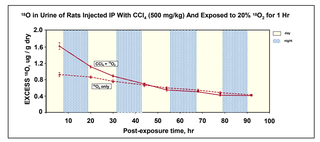
- Oxidative stress in mild asthmatics has been measured through a decrease in ascorbate concentrations in induced sputum. [5]



- Oxidative stress in asthmatic children was shown to be ameliorated through antioxidant supplements. [6]
- Non-invasive biomarkers of oxidative stress for use in human epidemiology have been identified in studies with rats:
  - -- Carbon tetrachloride induced oxidative stress to the liver was detectable in blood plasma and urine through measurements of isoprostanes, malondialdehyde adducts and GSSG/GSH ratios. [7]
  - -- Most blood plasma antioxidants were not good biomarkers due to interferences caused by liver spillage of antioxidants. [7]
  - -- Liver cell rupture assays measured injury more sensitively than direct oxidant adduct measurements. [7]
  - -- Ozone induced injury to the lung was not detectable by any blood plasma or urine measurement that was effective for carbon tetrachloride, however.
  - -- Oxygen-18 labeling made possible detection of excreted ozone adducts in the urine.
  - -- Oxygen-18 labeling also indicated that ozone adducts are not recycled by the body, but are completely excreted within about four days.



-- The use of oxygen-18 appears to make possible comparison of total-body oxidative stress between carbon tetrachloride and ozone, and also suggest the potential for finding even better non-invasive biomarkers.



IMPACTS:

- Finding biomarkers for oxidative stress will assist in future epidemiology studies.
- Quantification of oxidative stress will enable scientific extrapolation of animal toxicity data to humans.
- A better understanding of the role of antioxidants in modifying injury will enable improved risk assessments and better protection of susceptible individuals.
- *In vitro* model systems may reduce the number of experimental animals needed for toxicity studies.

FUTURE STUDIES

- Dose response modeling of chlorine gas for an Air Toxics studies involving human, animal and *in vitro* comparisons.
- Dosimetry and toxicity assessment of chlorine dioxide gas as part of a new Homeland Security study (see other poster).
- Dosimetry and toxicity assessment of "sentinel" species for Homeland Security (see other poster).
- Dosimetry and toxicity study of ingested bromate for the Water Program.
- Continuation of the Biomarkers of Oxidative Stress Study using endotoxin as the oxidative stressor.
- Two goal 8 studies examining blood plasma and tissue concentrations of antioxidant substances.
- External collaborations involving antioxidant pharmacokinetics and age susceptibility to ozone.

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SOLVING AGENCY PROBLEMS